TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (1989) 83, 586–588

Susceptibility of *Plasmodium falciparum* strains to chloroquine and mefloquine in the Amazonas Federal Territory of Venezuela

Marc Maynadié[1], Carmelina Peceño[2], Pedro-Luis Noriega[2] and Luis Yarzabal[2] [1]*Laboratoire d'Hématologie, Centre Hospitalier Universitaire du Bocage, 2 Bd Maréchal de Lattre de Tassigny, 21034 Dijon, France;* [2]*Centro Amazonico para Investigacion y Control de Enfermedades Tropicales, Apartado Postal 59, Puerto Ayacucho 7101, TFA, Venezuela*

Abstract

The susceptibilities of 27 *Plasmodium falciparum* strains to chloroquine and mefloquine were studied in the area of Puerto Ayacucho, Amazonas Federal Territory of Venezuela, to determine their levels of resistance *in vivo* and *in vitro*. 50% of these strains showed chloroquine resistance *in vivo*. No grade III chloroquine resistance was found. 25% of the strains were resistant to chloroquine *in vitro* and 9% were resistant to mefloquine *in vitro*. Preliminary results suggest that strains resistant to Fansidar® may also be found.

Introduction

Malaria remains one of the major public health problems in the world because of its extensive geographical distribution and the appearance of strains of *Plasmodium falciparum* resistant to the currently available antimalarial drugs (BRUCE-CHWATT, 1980). Chloroquine resistance was first reported in Venezuela by MABERTI (1960), and soon after it was reported from other South American countries (MOORE & LANIER, 1961) and south-east Asia (CONTACOS *et al.*, 1963), and, later, Africa (CAMPBELL *et al.*, 1979).

Since 1960, very few studies on resistant *P. falciparum* strains have been carried out in Venezuela (GODOY *et al.*, 1977), and none has been reported from the Amazonas Federal Territory (TFA).

This territory of 178 095 km^2 (ANONYMOUS, 1979) in the south of Venezuela is bordered on the east and south by Brazil and on the west by Colombia. In 1986, its population was estimated at 73 804 persons (OCEI, 1983), 0·4% of Venezuela's total population. However, 11% of Venezuela's malaria cases occur in TFA, 38% of which were *P. falciparum* infections with a morbidity rate of 1497/100 000 inhabitants, compared to 83·3/100 000 inhabitants for Venezuela as a whole (YARZABAL *et al.*, 1986).

The existence of resistant strains of *P. falciparum* was suspected because of the number of therapeutic failures observed with chloroquine treatment (WHO, 1973). This suspicion was supported by the results of resistance studies conducted in neighbouring Amazonian countries, Colombia (ESPINAL *et al.*, 1985) and Brazil (FERRARONI *et al.*, 1981).

The present study was undertaken to determine the levels of resistance *in vivo* and *in vitro* of *P. falciparum* strains to chloroquine and mefloquine in TFA.

Materials and Methods

Selection of patients

Twenty-two patients were studied, from Atures Department, TFA, in southern Venezuela (0°40'–26°15'N, 63°20'–67°52'W), where 42% of the Territory's population live and of which Puerto Ayacucho is the capital city. Patients were selected from those who presented voluntarily at Zone XIX of the Direccion General Sectorial de Malariologia y Saneamiento Ambiental (DGSMSA) or at the José Gregorio Hernandez hospital of Puerto Ayacucho, TFA, with febrile symptoms, or from those detected during our research group's expeditions in 1987. Only patients infected with *P. falciparum* with parasite densities ≥1000 asexual forms/mm^3 of blood, and who had not received antimalarial drugs during the previous 14 days (BRUCE-CHWATT, 1981), were included. The absence of previous antimalarial treatment was checked by the Dill-Glazko urine test (LELIJVELD & KORTMAN, 1970).

In vivo *test*

The selected patients were treated with chloroquine (Nivaquine®; Specia, France), giving a total dose of 25 mg/kg distributed in 3 oral doses as follows: 10 mg/kg on days 0 and 1, and 5 mg/kg on day 2 (WHO, 1973). The patients were observed during the hour following dosing to detect possible secondary effects; if the patient vomited, he or she was excluded from the study. Chloroquine assimilation was checked by the Dill-Glazko urine test (LELIJVELD & KORTMAN, 1970) on days 1 and 2.

The parasitaemia of 18 patients was monitored using the WHO 28 d extended field test and, for other patients, the 7 d alternative standard test (WHO, 1973). In both cases, a thick film was made daily on days 0–7, and on days 14, 21 and 28 in the 28 d test. After staining with Giemsa's stain, the asexual parasites and white blood cells were counted in 200 microscopic fields (×1000), and the parasite density per mm^3 was calculated assuming a mean of 8000 leucocytes/mm^3 of blood. The patients in whom asexual forms persisted until day 7 were routinely treated with a single dose of the sulfadoxine (500 mg)–pyrimethamine (25 mg) drug combination (Fansidar®, Spic, People's Republic of China) according to the WHO schedule (BRUCE-CHWATT, 1981). The susceptibility of these strains was defined according to the WHO criteria (WHO, 1973).

In vitro *test*

The sensitivity of *P. falciparum* strains *in vitro* to chloroquine and to mefloquine was evaluated by the microtechnique described by RIECKMANN *et al.* (1978). Chloroquine concentrations studied were 1, 2, 4, 5·7, 8, 16 and 32 pmol/litre; mefloquine concentra-

tions were 0·5, 1, 2, 4, 5·7, 8 and 16 pmol/litre. Each assay was performed in duplicate. Samples of 0·2 ml of venous blood were withdrawn with syringes containing 0·03 ml of anticoagulant solution (g/litre: citric acid 3·2, sodium citrate 25·8, dibasic sodium phosphate 2·18, glucose 25). Samples were then transferred to tubes containing 1·8 ml RPMI 1640 medium (Gibco, USA) supplemented with glucose 2 g/litre, *N*-tris(hydroxymethyl) methyl-2-aminoethane sulphonic acid (TES) 3 g/litre, gentamycin (Sigma, USA) 0·3 g/litre, L-glutamine 0·3 g/litre and pyruvic acid 0·11 g/litre. 25 µl of each sample were placed in each well of the test plate. After 26–30 h incubation in a candle-jar at 37°C, a thick film was made from each well and Giemsa-stained. The number of schizonts with more than 2 nuclei among 200 asexual parasites was counted. Parasite development at concentrations ≥5·7 pmol/litre of chloroquine or ≥4 pmol/litre of mefloquine indicated resistance to these drugs according to the WHO criteria (WHO, 1982).

Results

In vivo *test*

Twenty-two tests were performed: 8 strains (36·3%) were sensitive to chloroquine; 3 (13·6%) were sensitive/resistant; 6 (27·2%) showed RI resistance; 5 (22·7%) showed RII resistance; and none showed RIII resistance.

In vitro *test*

Eighteen micro-tests were performed; 6 did not give a valid result in the chloroquine susceptibility test, and 7 failed to give an acceptable result in the mefloquine susceptibility test (WHO, 1982).

Nine strains (75%) were sensitive to chloroquine, and 3 were resistant (25%). The sensitive strains were inhibited at a mean concentration of 5·51 pmol/litre.

Ten strains (90·9%) were sensitive to mefloquine, and one (9·09%) was resistant. The sensitive strains were inhibited at a mean concentration of 2·5 pmol/litre.

Table. Correlation of susceptibility of *Plasmodium falciparum* strains to chloroquine and mefloquine *in vivo* and *in vitro*[a]

Patient	Susceptibility to chloroquine *in vivo*	*in vitro*	Susceptibility to mefloquine *in vitro*
14	RII	R	S
25	S	S	S
35	S	S	ND
40	S	S	S
41	S	S	S
44	RII	R	R
64	RI	S	S
93	S/RI	S	S
100	RI	S	S
120	RI	R	S

[a]R=resistant, S=sensitive, ND=not determined.

The correlation between the results *in vivo* and *in vitro* is shown in the Table. All strains which were sensitive *in vivo* were sensitive *in vitro* to both chloroquine and mefloquine. Strain 93, for which the *in vivo* test did not allow discrimination between sensitive and RI, was also sensitive *in vitro* to both drugs. All but one strain (120) showing RI resistance *in vivo* were sensitive *in vitro*. All the strains RII resistant *in vivo* were also resistant *in vitro* to chloroquine, but only one (strain 44) was resistant to mefloquine *in vitro*. Strain 44 also showed resistance *in vivo* to Fansidar®; an *in vitro* test for this drug was not available in our laboratory at the time of this study.

Discussion

Since 1945, a malaria eradication programme has eradicated the illness from more than two-thirds of the country (GABALDON, 1983). TFA has always constituted an obstacle to the full success of this programme for geographical and sociological reasons, and malaria is still endemic in this region.

The problem became worrying with the resurgence of incidence of malaria since 1983 in Bolivar state of Venezuela, in the north of TFA, where it had been almost eradicated (GABALDON, 1983; NAVARRETTE *et al.*, 1986).

Furthermore, *P. falciparum* resistant strains in neighbouring regions and therapeutic failures suggested the presence of resistance in TFA. In Brazil (FERRARONI *et al.*, 1981), in Colombia (Florez, 1983, unpublished report), and in Bolivar state of Venezuela (NAVARRETTE *et al.*, 1986), the resistance rate reported ranged from 75% to 85% with 50% RI, 25% to 30% RII and 5% RIII. The rate we found was somewhat lower, with 27% RI, 23% RII and no RIII. The small number of cases we studied may explain these differences. No comparison can be made with other reports from TFA because of the absence of any previous study.

In all but 2 cases of resistance (patients 44 and 66), the infection was cured with Fansidar®. One of the patients was excluded from the study because of lack of compliance. Patient 44, a 5 year old boy, received a Fansidar® tablet on day 7 without any curative effect being detected by day 14. Three tablets were then given over the 3 following days, and the infection was cured. This result might be explained by either incomplete drug absorption or genuine resistance to Fansidar®. If it is the latter, it would be the first case reported for TFA. Although Fansidar® is used carefully (GABALDON *et al.*, 1971), reports of resistant strains already exist from nearby countries such as Surinam (OOSTBURG & JOZEFZOON, 1983), Colombia (ESPINAL *et al.*, 1985) and Brazil (DA SILVA *et al.*, 1984); resistance has even been observed in other areas of Venezuela (GODOY *et al.*, 1977).

Resistance to chloroquine *in vitro* was observed in 3 of 12 cases, and the strains were also resistant *in vivo* (Table). However, the *in vivo* resistance of strains 64 and 100 was not detectable *in vitro*. This was probably not due to poor chloroquine assimilation, as the Dill-Glazko urine test was positive. This apparent contradiction between test results *in vivo* and *in vitro* may be a consequence of the lack of sensitivity of our *in vitro* kit. The results of the *in vitro* test for mefloquine resistance indicated one resistant strain. This is interesting because mefloquine treatment has not yet been introduced in Venezuela. However, similar cases have also been reported in Indonesia (HOFFMAN *et al.*, 1985), Brazil (LOPEZ-ANTUNANO & WERNSDORFER, 1979) and Colombia (ESPINAL *et al.*,

1985), where mefloquine treatment is not widely used. This phenomenon may arise, as other authors noted, from quinine cross-resistance (WEBSTER *et al.*, 1985). Mefloquine has a molecular structure similar to that of quinine (both are amino-alcohols), which has been used in those areas for several years (GABALDON, 1983). This fact remains disturbing, however, because mefloquine is one of the few drugs of last resort against multi-resistant *P. falciparum* strains.

In summary, the present study confirms the existence of chloroquine-resistant strains of *P. falciparum* in the area of Puerto Ayacucho, TFA, Venezuela. More than 50% of the strains studied were chloroquine-resistant; 25% of them showed RII resistance. These results also suggest the presence of strains of *P. falciparum* resistant to both mefloquine and sulfadoxine-pyrimethamine. Therefore, future resistance studies should include evaluation of chloroquine, mefloquine and Fansidar® to allow complete analysis of the pattern of resistance. Further studies may be necessary to delineate fully the resistance pattern, and thus to permit the provision of adequate malaria prophylaxis in this South American country.

Acknowledgements

This work was funded by CONICIT (Consejo Nacional de Investigaciones Cientificas y Tecnologicas de Venezuela) project No S1-1842. We thank the Zone XIX of Direccion General Sectorial de Malariologia y Saneamiento Ambiental for their collaboration. Dr A. Capron is particularly thanked for the training provided to M.M. in the Pasteur Institute, Lille, France, and the Services Culturels, Scientifiques et Techniques of the French Embassy in Caracas for making his stay in Venezuela possible. We are grateful to Mrs P. Beer and Mrs I. Petralanda for critical reading of the manuscript and helpful comments.

References

Anonymous (1979). *Atlas de la Region Sur*. Caracas: Codesur.

Bruce-Chwatt, L. J. (1980). *Essential Malariology*. London: William Heinemann Medical Books.

Bruce-Chwatt, L. J., editor (1981). *Chemotherapy of Malaria*, 2nd edition. Geneva: World Health Organization.

Campbell, C. C., Chin, W., Collins, W. E., Tentsch, S. M. & Moss, D. M. (1979). Chloroquine-resistant *Plasmodium falciparum* from East Africa. Cultivation and sensitivity of the Tanzania I/CDC strain from an American tourist. *Lancet*, **ii**, 1151–1154.

Contacos, R. G., Lunn, J. S. & Coatney, G. R. (1963). Drug resistant falciparum malaria from Cambodia and Malaya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **57**, 417–424.

Da Silva, A. R., Craneiro, E. W. B. & Dos Santos, H. J. (1984). Reposta dos plasmodios humanos aos antimalaricos na ilha de São Luis, Estado Maranhão, Brazil. *Revista do Instituto de Medicina Tropical de São Paulo*, **26**, 139–146.

Espinal, T. C. A., Cortés, G. T., Guerra, P. & Arias, R. A. E. (1985). Sensitivity of *Plasmodium falciparum* to antimalarial drugs in Colombia. *American Journal of Tropical Medicine and Hygiene*, **34**, 675–680.

Ferraroni, J. J., Speer, C. A., Hayes, J. & Suzuki, M. (1981). Prevalence of chloroquine resistant falciparum malaria in the brazilian Amazon. *American Journal of Tropical Medicine and Hygiene*, **30**, 526–530.

Gabaldon, A. (1983). Malaria eradication in Venezuela: doctrine, practice, and achievements after twenty years. *American Journal of Tropical Medicine and Hygiene*, **32**, 203–211.

Gabaldon, A., Mendicoa, T. & Catellani, O. (1971). La resistencia de los parasitos malaricos a las drogas en Venezuela. *Boletin Informativo de la Direccion de Malariologia y Saneamiento Ambiental, Ministerio de Sanidady Asistencia Social*, **9**, 22–46.

Godoy, G. A., Volcan, G. S., Guevara, R., Medrano, C., Castro, J. & Texeira, A. (1977). Venezuelan strains of *Plasmodium falciparum* resistant to sulfa and pyrimethamine as demonstrated by *in vitro* test. *Revista Latinoamericana de Microbiologia*, **19**, 229–231.

Hoffman, S. L., Rustama, D., Dimpudus, A. J., Punjabi, N. H., Campbell, J. R., Oetomo, H. S., Marwoto, H. A., Harun, S., Sukri, N., Heizmann, P. & Laughlin, L. W. (1985). RIII and type resistance of *Plasmodium falciparum* to combination of mefloquine and sulfadoxine/pyrimethamine in Indonesia. *Lancet*, **ii**, 1039–1040.

Lelijveld, J. & Kortman, H. (1970). The eosin colour test of Dill and Glazko: a simple field test to detect chloroquine in urine. *Bulletin of the World Health Organization*, **42**, 477–479.

Lopez-Antunano, F. J. & Wernsdorfer, W. H. (1979). *In vitro* response of chloroquine-resistant *Plasmodium falciparum* to mefloquine. *Bulletin of the World Health Organization*, **57**, 663–665.

Maberti, S. (1960). Desarollo de resistencia la pyrimetamina. *Archivos Venezolanos de Medicina Tropical y Parasitologia Medica*, **3**, 239–259.

Moore, D. V. & Lanier, S. E. (1961). Observations on two *Plasmodium* infections with an abnormal response to chloroquine. *American Journal of Tropical Medicine and Hygiene*, **10**, 5–9.

Navarrette, L., Garron, A., Flores, F. & Morales de Gallo, L. (1986). *Resistencia de* Plasmodium falciparum *a la cloroquina en el Estado Bolivar, Venezuela*. Caracas: Servicio de Endemias Rurales, Zona III, Ministerio de Sanidad y Asistencia Social, mimeographed document.

OCEI (1983). *Proyecciones de Poblacion 1950–2000*. Caracas: Oficina Central de Estadistica y Informatica.

Oostburg, B. F. J. & Jozefzoon, L. M. E. (1983). Fansidar resistant *Plasmodium falciparum* in Surinam. *Tropical and Geographical Medicine*, **35**, 243–247.

Rieckmann, K. H., Campbell, G. H., Sax, L. J.& Mrema, J. E. (1978). Drug sensitivity of *Plasmodium falciparum:* an *in vitro* microtechnique. *Lancet*, **i**, 22–23.

Webster, H. K., Thaithong, S., Pavanand, K., Yougvanitchit, K., Pinswasdi, C. & Boudreau, E. F. (1985). Cloning and characterization of mefloquine-resistant *Plasmodium falciparum* from Thailand. *American Journal of Tropical Medicine and Hygiene*, **34**, 1022–1027.

WHO (1973). *Chemotherapy of malaria and resistance to antimalarials: report of a WHO Scientific Group*. Geneva: World Health Organization, Technical Report No. 529.

WHO (1982). *Instructions for use of the microtest kit for the assessment of the response of* Plasmodium falciparum *to chloroquine and mefloquine* in vitro. Geneva: World Health Organization, document no. MAP/82-1.

Yarzabal, L., Badell, M. & Fuentes, R. (1986). Epidemiologia de la malaria en el TFA. II. Evolucion de la morbilidad malarica en el periodo 1981–1985. *Informe Annual de Actividades 1986*. Puerto Ayacucho, Venezuela: CAICET.

Received 13 July 1988; revised 27 February 1989; accepted for publication 29 March 1989